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Previous research has shown that the acoustic startle response, a simple reflex mediated by four synapses in the brainstem and spinal cord, can be increased when elicited in the presence of a light previously paired with a footshock. This "fear-potentiated startle effect" can be selectively blocked by drugs that decrease anxiety in humans as well as by lesions of the central nucleus of the amygdala, an area of the brain known to be critical for fear. This year we found that local infusion of N-methyl-D-aspartate (NMDA) selective antagonists such as AP5 or CPP completely block the acquisition of fear-potentiated startle. This effect could not be attributed to a decrease in shock sensitivity or vision and did not occur when these compounds were infused into the cerebellum. These data indicate that an NMDA-dependent mechanism in the amygdala is involved in fear conditioning and that fear-potentiated startle may provide an excellent behavioral model system to analyze cellular and biochemical mechanisms of learning and memory.

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AFOSR - PROGRESS REPORT

MICHAEL DAVIS - YALE UNIVERSITY

JULY 1, 1988 - JUNE 30, 1989

Research Objectives

Previous research has shown that the acoustic startle response, a simple reflex mediated by four synapses in the brainstem and spinal cord, can be increased when elicited in the presence of a stimulus previously paired with a footshock. This "fear-potentiated startle effect" can be selectively blocked by drugs that decrease anxiety in humans as well as by lesions of the central nucleus of the amygdala, an area of the brain known to be critical for fear. A major goal of the work supported by the AFOSR is to determine the site of plasticity in the brain that mediates fear conditioning. Experiments during the last year have focused on the question of whether local infusion into the amygdala of drugs known to alter learning will affect the acquisition of fear-potentiated startle and how the peptide corticotropin releasing factor (CRF) might affect startle.

Accomplishments and progress

Local infusion of NMDA antagonists into the amygdala blocks fear conditioning

We have found that local infusion into the basolateral nucleus of the amygdala of the non-selective glutamate antagonist, λ-D-glutamylglycine, completely blocks the acquisition of fear-potentiated startle. λ-D-glutamylglycine was chosen because it blocks neural transmission at synapses which use excitatory amino acid transmitters such as glutamate and aspartate. Because it blocks transmission only temporarily (e.g., for an hour or so), it effectively produces a reversible "lesion" of brain structures that use excitatory amino acid transmitters. The fact that it completely blocked fear conditioning under conditions in which animals were tested one week later, when the drug effect would have completely worn off, provided our first critical evidence that excitatory amino acid transmission in the amygdala is involved in the learning of fear. Most importantly, however, we have also found that local infusion into the basolateral amygdala of selective N-methyl-D-aspartate (NMDA) antagonists (AP5 or CPP) also completely blocks the acquisition of fear-potentiated startle. Moreover, local blockade of NMDA receptors in the amygdala did not prevent animals from showing normal reactions to footshock, indicating that the interference with learning cannot be ascribed to a decrease in shock sensitivity. In addition, infusion of AP5 into the amygdala did not alter vision, because rats so treated still had marked visual pre-pulse inhibition, one objective measure of vision in rats. Finally, local infusion of AP5 into the cerebellum did not block the acquisition of fear-potentiated startle, even at a dose 8 times higher than the dose which completely blocked acquisition after infusion into the amygdala. The results indicate that fear-potentiated startle may provide an excellent model system to evaluate how NMDA channels regulate learning and

memory using a behavioral measure.

Lesions of the central nucleus of the amygdala block the excitatory effects of corticotropin releasing factor (CRF) on startle

Intraventricular infusion of the peptide corticotropin releasing factor (CRF) is known to produce a variety of behavioral effects which occur during periods of fear or stress. Consistent with previous reports, we have found that intraventricular infusion of CRF causes a marked increase in the amplitude of the startle reflex. By testing for a long period after infusion (e.g., 2-4 hrs) we have found that the effects of CRF are much greater than previously realized and last for a very long time (at least 6-8 hrs but not 24 hrs). Moreover, the effects of CRF given intraventricularly are eliminated by small bilateral lesions of the central nucleus of the amygdala. However, the amygdala does not seem to be the primary site of action of CRF because local infusion of CRF into the amygdala does not increase startle. In contrast, infusion of CRF into the parabrachial nucleus causes a marked and immediate increase in startle amplitude in doses 100 times lower than doses used intraventricularly. Because the parabrachial nucleus projects heavily and directly to the central nucleus of the amygdala, which in turn projects directly to the acoustic startle pathway, we believe that CRF given intraventricularly activates cells in the parabrachial nucleus which then leads to an increase in startle via parabrachial to amygdala connections which then project down to the startle pathway.

Future experiments

NMDA receptors and fear conditioning

1. At the present time, our fear conditioning phase consists of two, 45-min training sessions on two consecutive days using 10 light-shock pairings on each day. In order to completely block fear conditioning, the drug, which is infused 5 min before each test day, has to last for about 50 min, in order to block NMDA receptors for the entire training session. If, however, we could confine our training session to a much shorter total period of time, then the drug effect would only have to last for a short period of time, allowing a substantial reduction of the dose, thereby improving our ability to localize the critical site of action of the drug. Pilot data indicate that we can get good fear-potentiated startle by giving a total of 5 training trials over a total time of 8 min. This will be replicated using various shock intensities and retention intervals to maximize the level of fear conditioning. Once optimal parameters are found, future studies will use this paradigm to see if substantially lower doses of NMDA antagonists can be used to block fear conditioning. If so, then the studies listed below will be carried out using these lower doses.

2. Fear-potentiated startle involves two phases. One, the acquisition phase, in which lights and shocks are paired and the animal learns to be fearful of the light. Two, the performance or expression phase, in which the effects of prior fear-conditioning are measured by an increase in the startle reflex when elicited in the presence of the light paired with shock in the

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acquisition phase. As stated above, NMDA antagonists block acquisition of fear-potentiated startle. However, at the present time we do not know whether NMDA receptors are involved in the expression of fear-potentiated startle. To test this, rats will be given an acquisition session without drugs but then tested one week later after local infusion into the amygdala of NMDA antagonists or other compounds such as CNQX which block non-NMDA excitatory amino acid receptors. In the hippocampus, most of the expression of long-term potentiation is due to a change in non-NMDA transmission, although a small percentage of long-term potentiation appears to involve NMDA transmission. Hence, it is simply an empirical question at this stage whether NMDA or non-NMDA transmission will predominate in the amygdala after fear conditioning. It is even possible that neither compound will block the expression of fear-conditioning since other transmitters could just as well be involved in the expression of fear (e.g., peptides, which are abundant in the amygdala).

3. At the present time we have only infused NMDA antagonists into the basolateral nucleus of the amygdala and the cerebellum. As mentioned above, infusion into the cerebellum had no effect, even when much higher doses were used. However, we do not know about other parts of the amygdala. Based on work looking at the effects of excitatory amino acid antagonists into the startle pathway, the data indicate that these compounds have very limited diffusion in the brain, consistent with their hydrophillic structures. We hope to determine this more precisely by infusing radioactive AP5 into the amygdala and measuring the degree of diffusion using autoradiography (this will be done in collaboration with Dr. Gallager, who routinely using this methodology). Our problem thus far has been obtaining radioactive AP5, since it is only made in England, in very limited quantities, and it is very hard to get radioactive material sent from England quickly enough to retain activity.

If we can use this methodology and do find limited diffusion, our first "control" area will involve local infusion of NMDA antagonists into the central nucleus of the amygdala. This nucleus receives heavy projections from the basolateral nucleus; projects directly to the startle pathway; and is critical for the expression of fear-potentiated startle (as well as fear measured by a variety of other tests). However, the central nucleus has a relatively low density of NMDA binding sites and hence may not actually be involved in the formation of long term plasticity, but instead be critical for connecting the output of the basolateral nucleus to the startle pathway. If infusion into the central nucleus has no effect, then infusion into other amygdala nuclei will also be done. Finally, other studies will infuse NMDA antagonists into other brain areas (e.g., hippocampus), which are known to be critical for other types of learning as a further test of anatomical specificity.

Local infusion of CRF into the parabrachial nucleus

As mentioned in earlier progress reports, a single footshock markedly increases the acoustic startle reflex for a long period of time (shock sensitization). This effect is completely blocked by lesions of the central nucleus of the amygdala or the connection between the amygdala and the startle pathway. We believe this means that footshock activates the amygdala and that

this action may be critical for the acquisition of conditioned fear.

Neurons in the spinal cord which are activated by noxious stimuli like footshock project to the parabrachial nucleus which contains a high density of CRF receptors. It is possible, therefore, that footshock releases CRF into the parabrachial nucleus which then activates the amygdala via heavy projections from the parabrachial nucleus to the amygdala. Pilot data indicate that local infusion of CRF into the parabrachial nucleus increases startle. This will be replicated using several doses of CRF. In addition, we will test whether lesions of the amygdala will block the excitatory effects on startle of local parabrachial infusion of CRF (as they block intraventricular CRF effects) and possibly whether local infusion of CRF antagonists will block the excitatory effects of intraventricular CRF. Finally, on the basis of this work, we will decide whether to test if local infusion of CRF antagonists into the parabrachial nucleus will block shock sensitization as well as the acquisition of potentiated startle.

Articles published based on this work:

Davis, M. Sensitization of the acoustic startle reflex by footshock. Behavioral Neuroscience, 1989, 103, 495-503.

Boulis, N.M. and Davis, M. Footshock induced sensitization of electrically elicited startle reflexes. Behavioral Neuroscience, 1989, 103, 504-508.

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Davis, M. The role of the amygdala and its efferent projections in fear and anxiety. In: Psychopharmacology of Anxiety, Oxford University Press, London, 1989, pp. 52-79.

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Davis, M., Schlesinger, L.S. and Sorenson, C.A. Temporal specificity of fear conditioning: Effects of different CS-US intervals on the fear-potentiated startle effect. Journal of Experimental Psychology: Animal

Behavioral Processes

Davis, M., Animal models of anxiety based on classical conditioning: The conditioned emotional response (CER) and the fear-potentiated startle effect. Pharmacology and Therapeutics

Davis, M. Neural systems involved in fear-potentiated startle. New York Academy of Sciences.

Articles submitted for publichation

Hitchcock, J.M. and Davis, M. An efferent pathway of the amygdala involved in conditioned fear as measured with the fear-potentiated startle paradigm.

Rosen, J.B., Hitchcock, J.M., Sananes, C.B, Miserendino, M and Davis, M. A direct projection from the central nucleus of the amygdala to the acoustic startle pathway: anterograde and retrograde tracing studies

Campeau, S. and Davis, M. Reversible neural inactivation by cryogenic blockade in anesthetized and freely behaving rats.

Boulis, N.M., Kehne, J.H., Miserendino, M.J.D, and Davis. Differential blockade of early and late components of acoustic startle following intrathecal infusion of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) or DL-2-amino-5-phosphonovaleric acid (AP5)

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Invited lectures delivered relevant to this work:

Neural substrates of fear and anxiety: the role of the amygdala. Invited symposium speaker, National Institute of Mental Health Conference on Modulation of Defined Neural Circuits, Sept. 19, 1988, Elkridge, Maryland.

Neural systems involved in fear conditioning. Invited symposium speaker, National Institute of Drug Abuse Technical Review Meeting, Sept. 28, 1988, Bethesda, Maryland.

Neural systems involved in fear and anxiety. Department of Psychology, Harvard University, Cambridge, Mass., Oct. 12, 1988.

Neural systems involved in fear conditioning. Nova Pharmaceutical Corp, Nov. 9, 1989, Baltimore, Md.

Neural systems involved in fear and anxiety. Invited Symposium speaker, American College of Neuropsychopharmacology, Dec. 14, 1988, Puerto Rico.

A neural systems approach to the analysis of fear and anxiety. An invited symposium (Chairman) for the Neuroscience Association at Dartmouth, Jan. 27, 1989, Hanover, New Hampshire. Pharmacological and anatomical analysis of the fear-potentiated startle effect (Symposium presentation).

Neural systems involved in fear and anxiety. Department of Psychology, University of Penn., March 16, 1989, Phil., Penn.

Neural systems involved in fear and anxiety. Department of Psychology, Rutgers University, March 17, 1989, Camden, New Jersey.

Limbic circuits I: The functional anatomy of conditioned fear. Invited lecturer, First International School of Neuroscience, April 14, 1989, Padua, Italy.

Neural systems involved in fear-potentiated startle. Department of Psychology, University of Delaware, May 16, 1989, Newark, Delaware.